SULFUR METABOLISM BY BACTERIA AND YEASTS

BY

VERA MATILDA HANAWALT

B. A., Milwaukee-Downer College, 1935

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AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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Emanation of hydrogen sulfide as a waste product during degradation of sulfur compounds by bacteria and yeasts is of theoretical interest because of its relation to metabolic activities of microörganisms, and it has practical significance when formation of hydrogen sulfide is used to differentiate between species which is important in taxonomy, detect spoilage in canned foods, check the sanitary condition of water and determine the cause of metallic corrosion in pipe lines.

Gayon first demonstrated evolution of hydrogen sulfide during bacterial decomposition of eggs in 1877. Practical aspects of formation of this gas were stressed by Shardiger to study water pollution and by Orlowski to differentiate organisms. Early work indicated formation of hydrogen sulfide probably was an inherent characteristic of specific microörganisms. Thus investigators were interested in devising a medium containing an available sulfur substrate and an indicator sensitive to hydrogen sulfide in order to detect hydrogen sulfide-producing microörganisms. Lead compounds, especially lead acetate, were among the first indicators used and iron salts including ferric chloride and ferric ammonium citrate were also widely employed. A soluble bismuth ammonium citrate complex was suggested as an indicator in a medium devised by Hunter and Crecelius, and still later Utermohlen and Georgi proposed addition of cobalt and nickel nitrates as an indicator system. A few studies have been made on various organic and inorganic sulfur compounds to determine which were suitable substrates for cleavage to sulfuretted hydrogen. Saski and Otsuka were the first investigators to examine different compounds as sources of sulfur. They noted bacteria could reduce cystine, free sulfur, sodium thiosulfate and sodium sulfite to hydrogen sulfide, but no gas was liberated from sodium sulfate or taurine. The most extensive work on this problem was undertaken by Tanner who tested a number of fluorescent bacteria and a few other forms and reported liberation of sulfuretted hydrogen from peptone, cystine and thiourea, whereas none formed from 2-thiohydantoin, sulfates, sulfites and taurine. The following year Tanner observed that yeast-like fungi utilized sulfur in peptones, cystine, thiosulfates, sulfates, sodium

taurocholate, potassium thiocynate and thiourea. Clark, who carried out an investigation on thermophilic bacteria, found that hydrogen sulfide formed from commercial peptones, cystine, flowers of sulfur, thiosulfates, sulfates and gelatin.

The manner by which microörganisms liberate hydrogen sulfide has been considered by some workers. A reducing enzyme, called *philothion,* extracted from yeast and containing labile hydrogen, was thought by Rey-Pailhade to form hydrogen sulfide from sulfur. Petri and Maasen ascribed reduction to the presence of nascent hydrogen. Heffter believed this reducing capacity was inherent in the sulfhydryl group of organic materials in cells. An autoxidizable substance found in yeast, muscle, and mammalian liver and similar to *philothion* was described by Hopkins. Tarr related the reaction to a specific enzyme and Fromageot and Moubacher confirmed this concept. The latest theory of Stekol and Ransmeier indicates desulfurization might be associated with the length of the carbon chain.

To date an entirely satisfactory medium in which to measure hydrogen sulfide production is not available and considerable conflicting data are in the literature regarding the best indicator to use to detect the gas. This investigation was designed to determine which sulfur-containing compounds possess the most readily available form of sulfur for hydrogen sulfide production by bacteria and yeasts and which indicators are most sensitive to the presence of the gas in media.

The general experimental procedure of this investigation was as follows: Bacteria and yeasts were grown in a medium free from any form of sulfur except a known concentration of the inorganic or organic sulfur-containing compound to be examined as a substrate. Each sulfur compound was tested in the presence of a series of different indicators to determine which was most sensitive in the presence of a certain sulfur compound. Bacterial cultures were incubated five days at 37°C prior to reading. Production of hydrogen sulfide was manifested by a brown or black discoloration of the media and the amount of darkening indicated to a certain degree the extent of reduction. Yeast cultures were incubated 30 days at room temperature before readings were recorded.

The 18 bacterial cultures used in this report were obtained from the Departments of Bacteriology and Animal Pathology and Hygiene except the *Salmonella* strains which were secured from the American Type Culture Collection, and they were as follows: *Aerobacter aerogenes, Bacillus subtilis, Eberthella typhosa,* two strains of *Escherichia coli, Proteus vulgaris, Staphylococcus albus, Staphylococcus citreus, Salmonella aertrycke, Salmonella choleraesuis, Salmonella enteritidis,* three strains of *Salmonella paratyphi, Salmonella schottmülleri, Salmonella typhimurium* and two strains of *Shigella paradysenteriae.* The 12 yeast cultures tested were part of the stock culture collection of the Department of Bacteriology and included the cultures: Burgundy wine yeast, Champagne yeast, a *Cryptococcus* species, *Monilia candida, Mycoderma lactis, Mycoderma monosa, Mycoderma vini, Oidium lactis, Saccharomyces anomolous, Saccharomyces* of Curtis, *Saccharomyces ellipsoideus* and *Torula datilla.*

The semi-solid medium in which the sulfur compounds were metabolized by the bacterial cultures contained the constituents listed below: asparagine which provided the nitrogenous material; the non-reducing carbohydrate mannitol, which served as a source of carbon instead of the reducing sugar glucose, which frequently precipitates out the indicator; dipotassium phosphate; calcium chloride; agar; and the vitamins, nicotinic acid, riboflavin, calcium pantothenate and thiamin hydrochloride. It was necessary to add accessory materials to obtain satisfactory growth of all organisms. The five indicators which were added to the media to detect hydrogen sulfide produced by bacteria included the compounds , bismuth ammonium citrate, ferric ammonium citrate, ferric chloride, cobalt and nickel nitrates and lead acetate. The basic medium in which the yeast cultures were tested was similar to that used for the bacterial cultures except the medium was solid, ammonium chloride provided nitrogenous material instead of asparagine, and the amount of mannitol was greatly increased. Only two indicators, ferric chloride and lead acetate, were used to observe hydrogen sulfide production by yeasts. A series of control tubes were inoculated and incubated along with the test media to determine which indicators were precipitated out in the absence of any sulfur compounds.

Results of experimental work showed that among six peptones examined Wilson peptone provided the greatest amount of sulfur for bacteria. Fourteen bacterial and six yeast cultures produced hydrogen sulfide from l-cystine. Cysteine hydrochloride, which was reduced to hydrogen sulfide by 16 bacterial and 12 yeast cultures, surpassed all other compounds as a source of sulfur, and ferric ammonium citrate and ferric chloride were the most sensitive indicators in the presence of this amino acid. Only traces of hydrogen sulfide were formed from gelatin by eight bacterial cultures. Bismuth ammonium citrate and lead acetate were the most sensitive indicators in a medium in which flowers of sulfur served as the source of sulfur. Nearly all the bacteria reduced flowers of sulfur to sulfuretted hydrogen. Five bacterial cultures formed traces of hydrogen sulfide from sodium sulfate but none of the strains of yeasts reduced this salt. Fifteen bacterial cultures produced hydrogen sulfide in a concentration of 0.3 per cent sodium sulfite, but a concentration of two per cent proved toxic to both bacteria and yeasts. Sodium thiosulfate was not a good sulfur source for either bacteria or yeasts under conditions of this experiment. Small amounts of hydrogen sulfide were formed by bacteria in media containing thiourea and diphenylthiourea. Neither bacteria nor yeasts could utilize sulfur in dl-methionine for production of hydrogen sulfide. No sulfuretted hydrogen was formed by bacteria in media containing taurine, methyl-iso-thiourea sulfate, sodium thiocyanate, guanidine sulfate, aminoguanidine sulfate, sodium phenolsulfonate, thiolactic acid, sodium taurocholate, sulfanilic acid, ethyl sulfide and benzene sulfonic acid. The indicator bismuth ammonium citrate, though superior on the basis of total number of positive cultures detected, possessed the disadvantage of being precipitated out both by bacteria and yeasts. Iron salts were superior indicators in that they produced a more pronounced darkening of the medium. Cobalt and nickel nitrates were satisfactory indicators only when cysteine hydrochloride was the sulfur substrate.

The problem of hydrogen sulfide formation by bacteria and yeasts is a complex one involving many factors, some of which are: indicator used, sensitivity of indicator at a certain hydrogen-ion concentration, color reaction, toxicity and solubility of in-

dicator, ability of bacteria to cleave sulfur substrates, concentration of substrate, composition of medium, and metabolic end products liberated in the medium. Before one can state that the production of hydrogen sulfide is a specific property of certain microörganisms it is necessary to specify all factors involved.

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